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Immunomodulation by poly-YE reduces organophosphate-induced brain damage

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ABSTRACT

Accidental organophosphate poisoning resulting from environmental or occupational exposure, as well as the deliberate use of nerve agents on the battlefield or by terrorists, remain major threats for multi-casualty events, with no effective therapies yet available. Even transient exposure to organophosphorous compounds may lead to brain damage associated with microglial activation and to long-lasting neurological and psychological deficits. Regulation of the microglial response by adaptive immunity was previously shown to reduce the consequences of acute insult to the central nervous system (CNS). Here, we tested whether an immunization-based treatment that affects the properties of T regulatory cells (Tregs) can reduce brain damage following organophosphate intoxication, as a supplement to the standard antidotal protocol. Rats were intoxicated by acute exposure to the nerve agent soman, or the organophosphate pesticide, paraoxon, and after 24 h were treated with the immunomodulator, poly-YE. A single injection of poly-YE resulted in a significant increase in neuronal survival and tissue preservation. The beneficial effect of poly-YE treatment was associated with specific recruitment of $CD4^+$ T cells into the brain, reduced microglial activation, and an increase in the levels of brain derived neurotrophic factor (BDNF) in the piriform cortex. These results suggest therapeutic intervention with poly-YE as an immunomodulatory supplementary approach against consequences of organophosphate-induced brain damage.

1. Introduction

Nerve agents (e.g., soman, tabun, sarin, cyclosarin, and VX) are organophosphorous compounds that act by inhibiting the enzyme acetylcholinesterase, and are considered to be the most toxic of all chemical weapons. Their neurotoxic mechanism is also shared by organophosphate pesticides, which are one of the most common products of the modern chemical industry. Large scale release of organophosphate pesticides to the environment and their high availability frequently lead to intoxications, which result in long term morbidity and even in death. The widespread use of these compounds and their high neurotoxicity, not only constitutes a hazard of everyday life, but also establishes organophosphate pesticides, along with nerve agents, as a potential weapon (Colosio et al., 2003; Hoffman et al., 2007; Okumura et al., 1996; Sidell et al., 2008). To date, no treatments aimed at counteracting secondary degeneration following organophosphate poisoning are available (Layish et al., 2005; Markel et al., 2008; Sidell et al., 2008). While having no direct effect on survival of the patients, such treatments might be particularly important for counteracting organophosphate-induced cognitive and psychopathological impairments (Jamal et al., 2002; Levin et al., 1976; Rosenstock et al., 1991; Stallones and Beseler, 2002).

Organophosphate-induced brain injury is known to activate microglia (Zimmer et al., 1997). For decades, microglial activation was considered to be destructive in the context of the central nervous system (CNS) tissue, contributing to the secondary degeneration following injury (Liu et al., 2002). However, it was subsequently demonstrated that if microglial cells are properly regulated by T cell-derived cytokines or by cytokines released from blood-derived macrophages, they can support buffering of excessive levels of glutamate, secrete neurotrophic factors, induce neurogenesis, and promote tissue repair (Beers et al., 2008; Butovsky et al., 2006; Chiu et al., 2008; Ekdahl et al., 2009; El Khoury et al., 2007;

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Shaked et al., 2004; Simard et al., 2006). Specifically, in acute CNS insult, termination of the microglial activity via the recruitment of interleukin (IL)-10-secreting monocytes induced by T-cell based immunization was shown to be beneficial for recovery (Shechter et al., 2009). Moreover, the spontaneous recovery from CNS insults and the ability to cope with mental stress was shown to be T cell-dependent (Cohen et al., 2006; Kipnis et al., 2004b; Lewitus and Schwartz, 2009; Moalem et al., 1999; Yoles et al., 2001). Since basal immune maintenance is not sufficient to counteract the forces that drive neurotoxicity, boosting the protective T-cell response can be accomplished either by active immunization with the relevant self CNS antigens or via modulation of the naturally occurring T regulatory cells (Tregs), which constitutively suppress potential anti-self helper ($CD4^+$) T cells (Kipnis et al., 2002; Schwartz and Ziv, 2008). One way to achieve this aim is by administration of poly-YE, a high-molecular-weight copolymer (22–45 kDa) of glutamate and tyrosine with immunomodulatory properties (Cady et al., 2000; Vidovic and Matzinger, 1988), which is able to down-regulate the suppressive properties of Tregs (Ziv et al., 2007). This prompted us to test the neuroprotective potential of poly-YE in a model of soman-induced brain damage, and to further characterize the neuroimmune effects of poly-YE in a model of intoxication with paraoxon, a highly toxic metabolite of the organophosphate pesticide, parathion.

2. Materials and methods

2.1. Animals

For the paraoxon studies we used inbred male Sprague Dawley rats (12 weeks old), weighing 275–350 g that were supplied by the Animal Breeding Center of The Weizmann Institute of Science. The experiments and procedures were approved by the Weizmann Institute's Animal Care and Use Committee. For the soman studies, adult male Sprague Dawley rats (Charles Rivers, Kingston, NY, USA), weighing 275–350 g, were used. The experimental protocol was approved by the Animal Care and Use Committee at the US Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Animal Welfare Act of 1966 (P.L. 89–544). The number of animals that were used to generate data presented in the figures was: Fig.1 $n = 3–7$; Figs. 2 and 3 $n = 3–6$; Fig. 4 $n = 4–5$; Fig. 5 $n = 3$; for the behavioral studies presented in Supplementary Fig. 1, 5–9 animals were used. The exact number of animals in each experimental group is indicated in the figure legends.

2.2. Soman and paraoxon intoxication

Soman and paraoxon (nerve agent and pesticide, respectively) are highly toxic compounds that warrant immediate or even pre-exposure administration of antidotes in order to prevent the animals' death (Kassa et al., 2008). Both of these compounds act in a similar way via inhibition of cholinesterases (Eddleston et al., 2009; Sidell et al., 2008). The accepted treatment protocol includes termination of exposure, establishing or maintaining ventilation if necessary, and administration of antidotes, including the use of atropine (a peripheral muscarinic antagonist), and an oxime (a reactivator of the organophosphate-inhibited cholinesterase) such as HI-6 or toxogonin (obidoxime). The oxime treatment is used to prevent the immediate lethal effects of organophospahte exposure and atropine is given in order to reduce peripheral secretions and enhance survival (Eddleston et al., 2009; Sidell et al., 2008).

The soman animal model used in this study was chosen by US Army Medical Research Institute of Chemical Defense as a lead animal model to test various medical treatments as it acts fast and is highly lethal (McDonough and Shih, 1993). Rats were pretreated intraperitoneally with the oxime HI-6 (125 mg/kg; synthesized by Stanford Research Institute, Palo Alto, CA, USA), and 30 min later, challenged with soman (180 μg/kg, administered subcutaneously; equivalent to $1.6 \times LD_{50}$; US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, USA) or saline. The dose of soman (180 μg/kg) is used since it has been shown to elicit seizure activity in 100% of animals tested (McDonough and Shih, 1993). These rats were injected intramuscularly with atropine methyl nitrate (2 mg/kg; Sigma–Aldrich) 1 min after challenge. Since such antidotes do not cross the blood–brain barrier at the concentrations used, in case of seizures a member of the benzodiazepine family (e.g. diazepam) should be added as an anticonvulsant agent to insure survival (Sidell et al., 2008). Seizure onset was visually determined in each soman-exposed animal and these animals received diazepam (10 mg/kg, intramuscularly; Hospira, Lake Forest, IL) 40 min after seizure onset; saline-challenged rats received the same dose of diazepam 40 min after the saline injection. The 10 mg/kg dose of diazepam, administered 40 min after the start of soman-induced seizures, does not stop seizure activity but it provides significant protection against the lethal effects of soman thus reducing the number of animals that would need to be exposed in experiments such as this. Details of this model have been published and it was previously used for evaluating anticonvulsant drugs (McDonough and Shih, 1993; Shih et al., 1991, 1999).

Paraoxon animal model is regarded as a relatively safe model for studying organophosphate poisoning, even though the compound is also highly toxic (Krutak-Krol and Domino, 1985), and it was adopted in various research laboratories (Eisenkraft et al., 2007; Rosman et al., 2011). For the paraoxon study, rats were injected intramuscularly with paraoxon (0.45 mg/kg; equivalent to $1.4 \times LD_{50}$; Sigma–Aldrich) in saline. Control rats received saline. After 1 min, paraoxon-challenged rats were injected intramuscularly with the antidotes atropine sulfate (3 mg/kg; Sigma–Aldrich) and the oxime toxogonin (obidoxime, 20 mg/kg; Merck) in saline. The dose of paraoxon (0.45 mg/kg) is used since it has been shown to elicit seizure activity in 100% of rats tested, while still enabling the survival of the majority of the animals (Rosman et al., 2011).

2.3. Poly-YE treatment

Poly-YE (1 mg/rat; Sigma–Aldrich) in Dulbecco's phosphate-buffered saline (PBS, Gibco) was injected subcutaneously at indicated times after the intoxication with soman or paraoxon. Control animals received PBS. The regimen was chosen based on the previously determined dosage that provided the most efficient neuroprotection in a model of ischemic stroke (Ziv et al., 2007).

2.4. Barnes maze and activity monitoring

The Barnes maze (Med Associates, St. Albans, VT) was a round tabletop 122 cm in diameter, 140 cm high, with 18 holes (9.5 cm diameter) arranged every twenty degrees around the outer edge (Barnes, 1979). One hole, the target hole, had a small, dark, recessed escape box under the hole. The maze was placed in a brightly lit room with a variety of distinct visual cues arranged on the walls around the maze. Each subject was tested four times per day for four consecutive days, with 15–25 min between daily trials. In the beginning of each trial, the rat was removed from the transport cage, placed in the center of the maze and covered with an opaque cylindrical start chamber. The trial began 30 s later with the removal of the start chamber cylinder. As the subject explored the maze and poked its head in different holes, the handler silently communicated the head poke locations (i.e. hole number 1,